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Aurora-A enhances malignant development of esophageal squamous cell carcinoma (ESCC) by phosphorylating β-catenin

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ABSTRACT

The Aurora-A gene encodes a serine/threonine protein kinase that is frequently overexpressed in several types of human tumors. The overexpression of Aurora-A has been observed to associate with the grades of differentiation, invasive capability and distant lymph node metastasis of esophageal squamous cell carcinoma (ESCC). However, the molecular mechanism by which Aurora-A promotes malignant development of ESCC is still largely unknown. In this study, we show that Aurora-A overexpression enhances tumor cell invasion and metastatic potential *in vitro* and *in vivo*. Furthermore, Aurora-A overexpression inhibits the degradation of β-catenin, promotes its dissociation from cell–cell contacts and increases its nuclear translocation. We also demonstrate for the first time that Aurora-A directly interacts with β-catenin and phosphorylates β-catenin at Ser552 and Ser675. Substitutions of serine residue with alanine at single or both positions substantially attenuate Aurora-A-mediated stabilization of β-catenin, abolish its cytosolic and nuclear localization as well as transcriptional activity. In addition, Aurora-A overexpression is significantly correlated with increased cytoplasmic β-catenin expression in ESCC tissues. In view of our results, we propose that Aurora-A-mediated phosphorylation of β-catenin is a novel mechanism of malignancy development of tumor.

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1. Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant tumors. Despite remarkable advances in diagnostic and therapeutic techniques, the invasive and metastatic stage of ESCC progression still represents the most formidable barrier to successful treatment. The development of ESCC is a multistep, progressive process, and the acquisition of genetic alterations in tumor cells is a hallmark of cancer progression (Lin et al., 2009; Qi et al., 2012). Thus, better understanding of the molecular alterations during ESCC occurrence and progression should greatly improve tumor control and prevention, and may also lead to better clinical treatment.

Aurora-A, a centrosome- and microtubule-associated protein, is a serine/threonine protein kinase (Katayama and Sen, 2010). The increased attention has now been focused on Aurora-A kinase because of its interesting role in tumorigenesis. It has been shown that amplification and overexpression of the Aurora-A occur in several types of human tumors (Bischoff et al., 1998; Jeng et al., 2004; Sen et al., 2002; Zhou et al., 1998). Ectopic expression of Aurora-A in murine fibroblasts as well as mammary epithelia induces centrosome amplification, aneuploidy, and oncogenic phenotype (Bischoff et al., 1998; Jeng et al., 2004). Further, Aurora-A overexpression is more frequently associated with higher grade, higher stage, and identified as an independent prognostic factor for overall survival in a variety of human cancers (Hamada et al., 2003; Jeng et al., 2004; Neben et al., 2004; Sen et al., 2002). Recently, we have reported that expression of Aurora-A protein is highly increased in ESCC. Moreover, overexpression of Aurora-A is shown to associate with the grades of tumor differentiation and invasive capability (Tong et al., 2004). Tanaka et al. (Tanaka et al., 2005) further demonstrate that upregulation of Aurora-A expression is correlated with distant lymph node metastasis of ESCC. These clinical studies suggest that Aurora-A overexpression is closely associated with the development of ESCC. However, direct evidence for a role of Aurora-A in tumor development and metastasis is lacking and the molecular mechanism for the promotion of ESCC development by Aurora-A is currently poorly understood.

β-catenin is an ubiquitously distributed protein with multiple functions, which plays a critical role in tumorigenesis and development through its effects on E-cadherin-mediated intercellular adhesion and Wnt/wingless pathway (Nelson and Nusse, 2004). In response to Wnt signals, GSK-3β (glycogen synthase kinase 3β) is inhibited and phosphorylation of β-catenin at Ser-33/Ser-37 sites is decreased, leading to its stabilization, accumulation. β-catenin then translocates to the nucleus as an activator of T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors to stimulate transcription of a variety of growth-related genes (Bienz, 2005; Bienz and Clevers, 2000; Saito-Diaz et al., 2013). Increased β-catenin-TCF/LEF-1 transactivation by enhanced β-catenin stability is found in a wide variety of human cancers (Morin, 1999; Zhou et al., 2005). However, no data are available to indicate whether Aurora-A regulates β-catenin in ESCC.

In the present study, we illustrated that Aurora-A overexpression promoted tumor cell invasion and metastatic potential *in vitro* and *in vivo*. We also elucidated that Aurora-A inhibited the degradation of β-catenin and promoted its dissociation from cell–cell contacts, nuclear translocation, and transcriptional activity upregulation by direct phosphorylating β-catenin at Ser552 and Ser675. Overall, these studies reveal critical role of Aurora-A which contributes to the malignancy development of ESCC.

2. Material and methods

2.1. *Reagents and plasmids*

The antibodies were from the following sources: anti-Aurora-A, anti-β-catenin, anti-GSK-3β, anti-Gadd45, anti-P-β-catenin, anti-PS33-β-catenin and anti-PS37-catenin antibodies were from Cell Signaling Technology; other antibodies were from Santa Cruz Biotechnology. pEGFP-Aurora-A and pEGFP-β-catenin were constructed by inserting their open reading frames into pEGFP-C1 vectors, respectively. Aurora-A shRNA vector was generated by inserting target sequence of Aurora-A into pGCsi-U6/neo/GFP vector. Myc-tagged β-catenin was made by inserting the open reading frame of β-catenin into pCS2-MT vector. GST-Gadd45, GST-GSK-3β, GST-Aurora-A, GST-β-catenin and GST-β-actin were constructed by inserting their open reading frames into pGEX-5X-1 plasmids, respectively. The β-catenin mutants were generated by site-directed mutagenesis and confirmed by sequencing.

2.2. *Cell culture and transfection*

The KYSE150 cell line was generously provided by Dr Shimada of Kyoto University (Shimada et al., 1992). KYSE180, Colo680 and EC9706 cell lines were stored in our laboratory. The ESCC cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum (FBS). Transient transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions, and the cells were analyzed 2 days after the transfection. In the stable transfection, Aurora-A overexpression clones in KYSE150 cells or knockdown clones in EC9706 cells were selected by G-418 sulfate (Invitrogen) for 10–14 days.

2.3. In vitro *migration and invasion assays*

Invasion and migration assays were carried out by using Transwell motility chambers and performed as previously described (Tong et al., 2004).

2.4. *Xenograft assays*

All animal experiments were performed in accordance with relevant institutional and national guidelines and regulations. The cells were injected subcutaneously in the axillary region of four-week-old immune-deficient mice (BALBC/C-nu/nu, Vital River Co.). Tumor volumes were calculated using the formula (length) $\times$ (width)^2/2. The mice were euthanized at the end of 12 weeks after injection and examined for subcutaneous tumor growth and metastasis development. Specimens

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